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Antihyperglycemic of Olive Leaf (*Olea Europaea* L.) Ethanol Extract in Obesity Diabetic Male Mice (*Mus musculus*)

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ABSTRACT
Olive leaf (Olea europeae) contains the primary polyphenols: oleuropein, hydroxytyrosol, luteolin 7-glucoside, apigenin 7-glucoside, and verbascoside. Empirically olive leaf is used for lowering blood sugar because it has antioxidant properties. This study aimed to determine the antihyperglycemic activity of olive leaf ethanol extract in obese male mice. This study was conducted by measuring fasting blood sugar by pre and post-administration of olive leaf ethanol extract. The method used in this study was the administration of a high-fat diet for six weeks and the alloxan induction method. The test group was divided into four groups (mg/KgBW) consisting of a control group (diabetic control and metformin 500) and group treatments of olive leaf extract (225 and 400). Each group consisting of five mice. Data analysis using Minitab 19. The results showed that there was an average difference between pre and post (mg/dL) in each group (P-Value <0.05) were BC (139.80±10.35) and (151.80±21.36), metformin (152.20±14.06) and (75.40±20.48), EOD 225 (157.80±16.90) and (99.20±30.60), EOD 400 (178.20± 35.10) and (80.60±20.79). It can be concluded that the ethanolic extract of olive leaf (Olea Europaea L.) in the treatment group can reduce fasting blood glucose levels of alloxan-induced obese male mice.

Keywords: *Antihyperglycemic, Olive Leaf, Alloxan, Extract, obesity*

1. INTRODUCTION

Type 2 diabetes mellitus is a metabolic condition with increased blood glucose levels caused by impaired insulin secretion due to cellular insulin resistance [1]. Patients with diabetes mellitus have increased blood glucose levels that exceed 200 mg/dL and have fasting blood glucose levels >125 mg/dl [2].

Type 2 diabetes has the leading risk factor is obesity which causes insulin resistance [3]. The main risk factor for type 2 diabetes is obesity which causes insulin resistance. Obesity is associated with lipid accumulation, especially in adipose tissue, resulting in adiposity dysfunction leading to macrophage infiltration and mild inflammation [4]. A good treatment for this feature is to provide two functions: lower blood sugar and control weight gain [5].

One of the plants that are useful as medicine is olive (Olea Europaea L.). This plant comes from the Mediterranean with a quite widespread in certain countries such as Greece, Italy, Spain, Portugal, and France [6]. Olives are used as traditional medicine in the Mediterranean zone. Natural products are used for various purposes, including reducing fever, inflammatory malaria, arrhythmias and relieving intestinal spasms [7].

The content of oleuropein in olive leaves allegedly inhibits the increase in blood glucose [8]. Oleuropein can also inhibit oxidative stress caused by hyperglycemia [9]. Oleuropein is the main phenolic constituent of leaves, olive oil, and fruits up to 14% of dry weight [10].

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2. MATERIAL AND METHODS

2.1. Plant Materials

The sample used in this study was the ethanolic extract of olive leaves (*Olea Europaea* L.). The ethanol extract of olive leaves (Olea Europaea L.) used in this study was purchased from MarkHerb Indonesia with 70% ethanol solvent extracted by the maceration method.

2.2. Experimental Animals and Ethics Statement

Twenty male albino mice weighing about 20 g were used as experimental animals in the present study were divided into four groups. Each group consisted of five mice. Before the study started, the test animals were acclimatized for seven days, fed, given drinking water, and fasted before treatment. Mice were maintained at a temperature (25 ± 5° C), humidity (55 ± 5%), and a cycle of 12 hours of dark/light with good ventilation. Procedure instructions for test animals under the provisions of the ethics committee that have been approved by the Bakti Tunas Husada Tasikmalaya ethics committee with an approval number No.062/kepk-bth/VII/2021.

2.3. Induction of Obesity

Obesity induction was maintained by feeding a high-fat diet for six weeks, weighing the mice once a week. The modified high fat composition consists of 80% pork pellets, 15% butter and 5% egg yolks [11][12]. The standard weight of mice is 20 grams, and the average weight of obese mice is >30 grams. Rats were obese with bodyweight >20% of initial weight [13]. Induced by feeding a high-fat diet as much as 100 grams/day of treatment [14].

2.4. Induction of Animal Model of Diabetes Mellitus

Alloxan monohydrate powder reconstituted for injection using sterile aquabidest. The intraperitoneal dose of alloxan used in this experiment was 175 mg/kg BW [13] or for mice weighing 20 grams was 0.455 mg/20gBW. Before the induction process by using alloxan, the test animals were fasted for 12 hours beforehand but still given drinking water. Alloxan monohydrate solution was injected intraperitoneally at a dose of 0.455 mg/20 gBW of group mice in the diabetic control, positive control, the treatment I, and treatment II groups, each of which consisted of 5 test animals for six days (on day 42 to day 49). After the injection, the mice were fed and drank as usual. Measure the fasting blood glucose levels of mice after alloxan induction and ensure that the mice have permanent hyperglycemia [15]. Mice have hyperglycemia if the increase in fasting blood glucose levels is 126 mg/dl [16].

2.5. Animal Grouping

Mice included in the experiment were randomly allocated into four groups as follows:

(i) Group 1: this group was considered as diabetic control. The mice in this group were given an equivalent volume of the vehicle (1% CMC)/day by oral gavage daily for seven days. They were fed a high-fat diet. The giving volume is adjusted.

(ii) Group 2: this group was considered as a positive control. Metformin dose of 500 mg/kg BW/day was converted into a mice dose and then dissolved in 1% Na-CMC by oral gavage daily for seven days. They were fed a high-fat diet. The giving volume is adjusted.

(iii) Group 3: The extract olive dose of 225 mg/kg BW/day (EOD255) was converted into a dose of mice and then dissolved in 1% Na-CMC by oral gavage daily for seven days. They were fed a high-fat diet and drank as usual. The giving volume is adjusted.

(iv) Group 4: The extract olive dose of 400 mg/kg BW/day (EOD400) was converted into a mice dose and dissolved in 1% Na-CMC by oral gavage daily for seven days. They were fed a high-fat diet and drank as usual. The giving volume is adjusted.

2.6. Sample Collection

Fasting blood glucose uptake before treatment on day 49 after administering alloxan (pre-test) and day 56 (post-test) after treatment. At the time of blood collection, the mice were fasted first but still given water. Before blood sampling, mice were cleaned first with 70% alcohol. Then take the blood through the tail of the mouse with a syringe. A drop of blood from the tails of mice dropped on the glucose strip included in the glucometer. Wait for 10 seconds to wait for the

result. Fasting blood sugar is checked using a glucometer. Record the value stated on the glucometer, which is the value of blood glucose concentration with units of mg/dL [17].

3. RESULTS AND DISCUSSION

3.1. Body Weight

The high-fat diet in all test groups experienced a significant increase in body weight during the 42-day induction phase (table 1). In this study, the fat given was higher than the amount of protein. High fat composition causes obesity. Obesity occurs due to an imbalance in food intake, basal metabolism, and energy expenditure. In this study, the administration of high fat caused an increase of > 60%. Mice usually will show an increase in body weight of 20-30% after four weeks, compared with rats fed food [18].

Nicholas et al. stated that ad libitium feeding a high-fat diet to rats would lead to excessive consumption, increasing body weight, especially in the light cycle [19]. Weight gain because a high-fat diet causes fat deposits under the skin [20]. Fat will be stored in adipose in neutral lipids, whereas in conditions of nutritional deficit, adipose tissue supplies nutrients to other tissues through lipolysis. [21].

At the time of treatment, the mice were still given a high-fat diet. There was a decrease in body weight in each test group after giving the dose of the test substance; both the metformin, EDO225, EDO400 groups experienced weight loss. While in the diabetic control group, they remained weight gain.

Table 1 Comparison of Body Weight Measurement of Pre and Post High-Fat Feeding groups (@ five mice)

Group	Body weight ($\bar{x} \pm SD$ (g))		p-Value	Percentage of weight gain (%)	Body weight after treatment ($\bar{x} \pm SD$ (g))	p-Value	Percentage of weight loss (%)
	Pre	Post					
Diabetic Control	23.60±1,52	39.40±1,67	0.00	60.6	40,20±3,42	0.161	-
Metformin	21.00±1,73	37.60±3,29		62.4	34,60±3,71		7.98
EOD 225	21.00±2,83	38.00±1,87		62	36,60±2,41		3.68
EOD 400	21.80±1,64	37.80±4,87		62.2	36,20±5,07		4.23

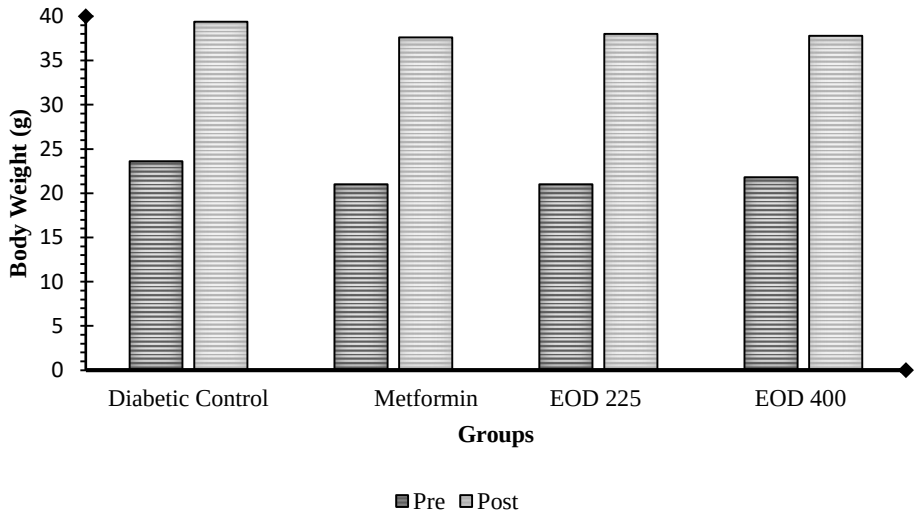

Figure 1 Pre and Post Body Weight

Metformin had a higher percentage of weight loss compared to the olive extract group in alloxan-induced obese mice. This weight loss is because metformin can reduce appetite in mice due to hypothalamic modulation, inhibition of incretin secretion and changes in intestinal microbial composition [22].

3.2. Blood Glucose

After alloxan induction, fasting blood glucose in mice increased in all groups in table 3 (pre). Alloxan induction results in a decrease in insulin production, causing diabetes in experimental animals [23]. Alloxan inhibits insulin secretion by reducing the oxidation of glucose in the process of glucokinase [24]. Alloxan is reduced to dialuronat acid is then subjected to reoxidation of the redox cycle and results in free radicals such as reactive oxygen species (ROS) [25]. Thus, oxidative stress partially damages pancreatic beta cells and reduces insulin production so that insulin cannot mediate blood glucose transport into cells [26].

Table 2 Comparison of the results of the fasting blood glucose measurements Pre and Post Treatment Groups (@ five mice)

Groups	Blood Glucose ($\bar{x} \pm SD$ (mg/dL))		p-Value
	Pre	Post	
Diabetic Control	139.80±10,35	151.80±21,36	0.00
Metformin	152.20±14,06	75.40±20,48	
EDO 225	157.80±16,90	99.20±30,60	
EDO 400	178.20±35,10	80.60±20,79	

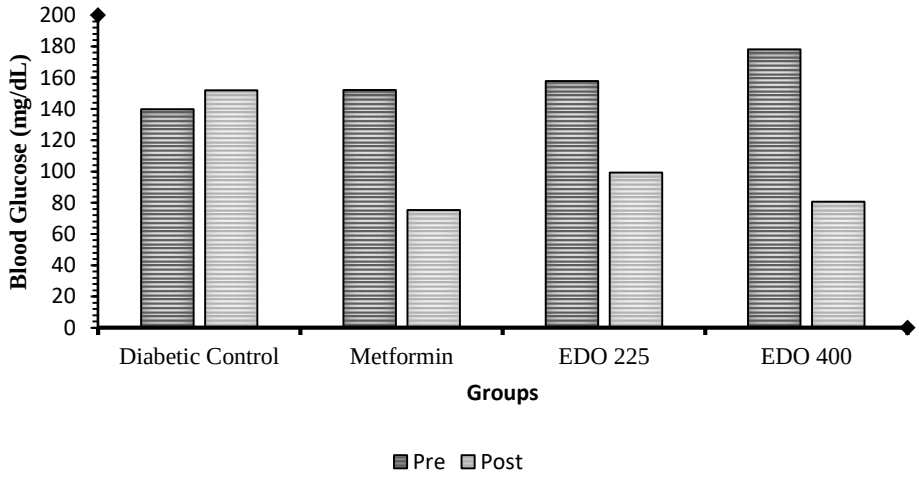

Figure 2 Fasting blood glucose pre and post-treatment Groups

Based on the results of comparative measurements of fasting blood sugar throughout the pre and post-treatment groups, there are differences in blood sugar levels. The pretreatment group of mice was hyperglycemic. While post-treatment metformin, EDO225, EDO400 decreased blood sugar. In this study, it was seen that the diabetic control group had glucose levels that tended to increase during treatment because they did not receive antidiabetic intake. Excess fat in obesity will cause an increase in glucocorticoid hormones. High glucocorticoid hormones cause lipophilicity, which triggers the emergence of gluconeogenesis in which glucose cannot be carried by cells and ultimately, the

gluconeogenic process converts liver protein into glucose and causes an increase in blood sugar [27].

Fasting blood glucose after administration metformin and extract (EEDZ 225 and 400 mg) both have a blood sugar lowering activity. Blood sugar levels after administration of the two extract doses did not differ as well as metformin administration.
Metformin administration in the diabetic obesity group acts on the gluconeogenic pathway [28]. At the molecular level, metformin inhibits the mitochondrial, decreases hepatic glucose production, and increases hepatic cytosolic redox state without altering hepatic triglyceride content or gluconeogenic enzyme expression [29].
All treatment groups that were given the extract experienced a decrease in blood glucose levels that previously experienced hyperglycemia so that it could be said that the extract affected fasting blood sugar. This treatment is due to the content of the ethanolic extract of olive leaves, namely oleuropein. Oleuropein has a distinctive structure that includes glucose residues to compete with glucose for transport through SGLT1 (sodium-glucose cotransporter 1) [30]. Oleuropein can also improve glucose tolerance and insulin resistance by modulating insulin secretion and inhibiting the formation of Reactive Oxygen Species (ROS) [31].
The two treatment groups of olive leaf ethanol extract (Olea Europaea L.) had a hypoglycemic effect. However, the most effective dose between the two treatment groups in reducing fasting blood glucose levels was 400 mg olive leaf ethanol extract because the decrease in fasting blood glucose levels in this group was at normal fasting blood glucose range <100 mg/dL [32]. Meanwhile, the decrease in fasting blood glucose levels in the 225 mg group treatment of olive leaf ethanol extract was in the range of pre-diabetic fasting blood glucose levels (impaired fasting glucose) of 100-125 mg/dL. [33]. The decrease in the treatment group at 400 mg/kg BW was higher than that at a 225 mg/kg BW dose. Metformin and olive leaf ethanol extract at a dose of 400 mg/kg BW have a similar effect in reducing fasting blood glucose levels in mice, and this is due to the presence of oleuropein in the extract so that olive leaf ethanol extract can be used as replacement therapy for metformin in the treatment of hyperglycemic.

4. CONCLUSION

Olive leaf ethanol extract (Olea Europaea L.) in the treatment group effectively reduced fasting blood glucose levels of alloxan-induced obese male mice .

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AUTHOR CONTRIBUTION

The authors confirm contribution to the paper as follows: study conception and design: Nurhidayati Harun; data collection: Elin Herlina and Siti Rahmah; analysis and interpretation of results: Nurhidayati Harun, Elin Herlina, and Siti Rahmah; draft manuscript preparation: Nurhidayati Harun and Elin Herlina. All authors reviewed the results and approved the final version of the manuscript.

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